Parasitism of the soybean aphid, *Aphis glycines* by *Binodoxys communis*: the role of aphid defensive behaviour and parasitoid reproductive performance

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Abstract

The Asian parasitoid, *Binodoxys communis* (Gahan) (Hymenoptera: Braconidae), is a candidate for release against the exotic soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), in North America. In this study, we examined preferences by *B. communis* for the different developmental stages of *A. glycines* and investigated consequences of these preferences for parasitoid fitness. We also determined to what extent aphid defensive behaviours mediate such preferences. We found that *B. communis* readily attacks and successfully develops in the different *A. glycines* developmental stages. *Binodoxys communis* development time gradually increased with aphid developmental stage, and wasps took longest to develop in alates. An average (±SE) of 54.01±0.08% of parasitized *A. glycines* alatoid nymphs transformed into winged adult aphids prior to mummification. No-choice assays showed a higher proportion of successful attacks for immature apterous *A. glycines* nymphs compared to adults and alatoid nymphs. Also, choice trials indicated avoidance and lower attack and oviposition of adults and alatoid nymphs. The different aphid stages exhibited a range of defensive behaviours, including body raising, kicking and body rotation. These defenses were employed most effectively by larger aphids. We discuss implications for the potential establishment, spread and biological control efficacy of *A. glycines* by *B. communis* in the event that it is released in North America.

Keywords: biological control, host quality, host selection, koinobiont parasitoids, fitness, life history, phoresy

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Introduction

Biological control introductions are being considered against the soybean aphid, *Aphis glycines* Matsumura, an invasive species from Asia that is a destructive pest of soybeans in North America (Heimpel *et al.*, 2004; Ragsdale *et al.*, 2004; Wu *et al.*, 2004; Wyckhuys & Heimpel, 2007;

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Wyckhuys *et al.*, 2007a,b). A strain of *Binodoxys communis* (Gahan) (Hymenoptera: Braconidae) that was collected from soybean aphid in China is one of the most promising natural enemies for release against *A. glycines*. This species appears to be very well adapted to *A. glycines* in laboratory studies and exhibits fairly high levels of host specificity (Wyckhuys *et al.*, 2007b; N. Desneux, personal communication).

The immature stages of insect parasitoids, such as *B. communis*, depend on their host for nutrients, and so adult female parasitoids must accurately assess the suitability of hosts for progeny development (e.g. Sequeira & Mackauer, 1992a; Colinet *et al.*, 2005; Henry *et al.*, 2005). Many parasitoids preferentially attack certain sizes, ages or stages of a given host species (Mackauer, 1973; Hopper & King, 1984; Liu *et al.*, 1984; Wang & Liu, 2002; Lin & Ives, 2003). These preferences influence developmental rate, survival and reproductive capacities of parasitoid offspring (Lewis & Redlinger, 1969; Nechols & Kikuchi, 1985; Hopper, 1986; Sequeira & Mackauer, 1993; Godfray, 1994; Lacoume *et al.*, 2006). Host-stage preferences can also strongly influence host behaviour and population growth (Hopper & King, 1984; Murdoch *et al.*, 1987; Murdoch *et al.*, 2003; Lin & Ives, 2003) and may affect parasitoid efficiency in biological control of aphids (Hågvar & Hofsvang, 1991).

All aphid parasitoids are endoparasitic koinobionts, meaning that eggs are laid within the host and the host continues developing after parasitism. Thus, the host stage used for oviposition generally precedes the host stage(s) used for larval development, complicating oviposition strategies (e.g. Kouamé & Mackauer, 1991; Sequeira & Mackauer, 1992b; Rivero, 2000; Li & Mills, 2004; Jenner & Kuhlmann, 2006). Koinobiosis also may decouple host stages that engage in defensive behaviour from those that support parasitoid development, in some cases allowing development on hosts that would otherwise be inaccessible (Liu *et al.*, 1984; Gerling *et al.*, 1990; Weisser, 1994, 1995; Chau & Mackauer, 2000). For example, although large aphids contain more nutritional resources than small aphids, they also defend themselves more effectively than smaller ones (Gerling *et al.*, 1990; Chau & Mackauer, 1997; Losey & Denno, 1998; Chau & Mackauer, 2000).

Differences in host-stage use also have implications for parasitoid dispersal and establishment in novel environments. Most aphid species, including *A. glycines*, exhibit a polyphenism whereby individuals exhibit both winged (alate) and wingless (apterous) morphs (Dixon, 1998; Ragsdale *et al.*, 2004; Hodgson *et al.*, 2005). Alates generally leave their natal host plant and colonize new plants, eventually in different environments. Successful parasitism of winged or alatoid morphs could then lead to situations where parasitoid eggs or larvae are carried for considerable distances within their host (Kelly 1917; Hight *et al.*, 1972; Rogers *et al.*, 1972; Rauwald & Ives, 2001). Such phenomena could be of great importance to establishment, spread and efficacy of potential biological control agents such as *B. communis*.

Here, we investigate *B. communis* parasitism of different developmental stages of the soybean aphid, *A. glycines*, under choice and no-choice settings. Parasitism differences are related to *A. glycines* defensive behaviour, *B. communis* handling costs and oviposition success on each of the *A. glycines* stages. We also assess parasitoid fitness on each of the *A. glycines* instars by measuring key life-history traits, such as development time, survival and sex ratio (e.g.

Roitberg *et al.*, 2001). This research increases basic knowledge of this host-parasitoid association and helps predict *B. communis* establishment, spread and biological control success upon release in North America.

Materials and methods

Study insects

We established a colony of *A. glycines* from individuals that were collected in 2003 from a soybean field in St Paul, Minnesota, USA. This colony has subsequently been maintained in the Minnesota Department of Agriculture/ Minnesota Agricultural Experiment Station (MDA/MAES) Quarantine Facility in St Paul with periodic supplementation of aphids from the field. As aphid colonies were never started from one single aphid and no genetic characterization was done, no estimates are available of the number of clones of which the *A. glycines* colony consisted. The *A. glycines* colony was kept on soybean plants (cultivar M96-D133151), which were grown under greenhouse conditions ($25 \pm 5°C$, 60–80% RH and L16:D8).

A Chinese strain of *Binodoxys communis* was initiated with seven males and 33 females from collections of parasitized *A. glycines* by K. Hoelmer, K. Chen and W. Meikle made in several soybean fields in late August 2002 near Harbin ($45°41'27''$ N, $126°37'42''$ E) and in Suihua County ($45°36'28''$ N, $126°57'49''$ E) in the Chinese province of Heilongjiang. Voucher specimens of progeny from the material collected in China are stored frozen in molecular grade ethanol at the USDA-ARS Beneficial Insect Introduction Research Laboratory in Newark, Delaware, USA. We maintained *B. communis* in three subpopulations on *A. glycines* with a geometric mean of 66–68 adult parasitoids for each subpopulation for 26 generations. The parasitoid colony at the MDA/MAES Quarantine Facility was initiated from a total of 102 mummies in 2003, and has since been maintained on *A. glycines*. For experiments, we collected *B. communis* mummies from soybean plants and isolated these in clear gelatin capsules (size 0; Drum Point Pharmacy, Brick, NJ, USA). Adult female wasps were mated within 24 hr of emergence and kept in capsules with a droplet of mixed-flower honey prior to their use in experiments.

No-choice parasitism trials

In the first experiment, we determined *B. communis* fitness on each of the *A. glycines* developmental stages by measuring overall parasitism levels as well as a set of life-history traits. Hodgson *et al.* (2005) reported *A. glycines* to have a total of four different immature instars, and winged or wingless adult stages. Third and 4th instar nymphs of winged morphs possess wing-pads and are termed alatoid nymphs; of these two morphs, we included 4th instar alatoids in our experiment, yielding a total of seven *A. glycines* types to be tested.

We planted soybean plants in the greenhouse and used them at the V1 stage for our experiments. The V1 developmental stage is characterized by a fully developed first trifoliate leaf and expanded unifoliate leaves (McWilliams *et al.*, 2004). In the laboratory, individually potted live plants were covered with a 0.5 l transparent PETE plastic cup (Solo Cup Company, Urbana, Illinois, USA) from which the bottom was removed. The top of the plastic cups was fitted

with a fine nylon mesh, and the entire unit will be referred to as an 'experiment cage'. We placed a total of 40 individuals of a given *A. glycines* stage onto each soybean plant using a fine brush. The different aphid stages were determined using an identification key developed by Hodgson *et al.* (2005). We allowed aphids to establish on plants for 1–2 hoursbefore the introduction of parasitoids.

Mated female *B. communis* were subsequently transferred to the cages and allowed to parasitize aphids for 4 h. The 4 h period ensured that high numbers of aphid offspring were not produced during the experiment, thereby likely distorting parasitism rates on each of the different aphid stages. Parasitoids were introduced into the cages between 12:00 and 14:00 and were removed after 4 h. The cages were maintained at 25°C, 75% RH and 16 : 8 L : D and checked on a daily basis for the presence of parasitoid mummies.

Mummies were counted upon formation, and the number of days until mummy formation was recorded. Mummies were subsequently placed singly in clear gelatin capsules (size 0) and the number of days until parasitoid emergence was recorded. The sex of emerged parasitoids was determined and sex ratio is expressed as the proportion of adults that were male. We report the parasitism rate as the number of mummies divided by the starting number of aphids (i.e. 40). Although this measure does not distinguish parasitoid acceptance of hosts from host physiological suitability for parasitoid development, it provides a useful assessment of the net effect of parasitoid choice and host suitability on overall parasitism success (Li & Mills, 2004; Colinet *et al.*, 2005). For each of the seven *A. glycines* developmental stages, we carried out a total of ten replicates.

To compare *B. communis* life history traits on the different *A. glycines* developmental stages, we used a Kruskal-Wallis test or One way analysis of variance (ANOVA) with Fisher's protected LSD as post-hoc analysis, according to the normality of the data set.

No-choice assay of host acceptance

A second experiment was done to quantify *B. communis* acceptance of each of the *A. glycines* developmental stages to determine the nature and extent of defensive behaviour of these stages. The behavioural arena consisted of a single leaflet that was removed from one of the fully expanded leaves of an uninfested V3–5 soybean plant and placed upside down within a 5.8 cm dia. Petri dish filled with moist sand. The leaflet had a diameter of >5 cm and commonly occupied the entire space within the Petri dish. The V3–5 soybean developmental stages are characterized by fully developed and expanded third-fifth trifoliate leaves (McWilliams *et al.*, 2004). The Petri dish was then placed under a Leica GZ6E dissecting microscope. On this leaflet, we placed one individual of a given *A. glycines* stage and allowed it to settle for 5 min. Aphids were collected with a fine brush from *A. glycines* colonies on soybean plants of identical phenological stage and (visually) similar quality (Stadler *et al.*, 1994). Next, a one-day-old, mated *B. communis* female was gently introduced into a 1 cm dia. × 0.65 cm high clear plastic dome. This dome was then placed over the individual aphid within the Petri dish. The observation was started when the parasitoid first encountered the aphid.

We observed both aphid and parasitoid behaviour until a successful oviposition occurred but not longer than 5 min. For *B. communis*, we recorded the time elapsed until oviposition, the number of encounters and the number of probing events. An encounter was defined as the parasitoid making contact with the aphid after having walked away from it for >5 s for re-encounters. All intervals were timed with a stopwatch to the nearest second. For the different *A. glycines* stages, we recorded defensive behaviour as 'kick', 'rotate', 'walk away' or 'cornicle secretion'. Kicking was defined as the aphid raising its body and then contacting the parasitoid with one of its legs. Exposures were replicated 25 times for each aphid stage. For every observation, a different *B. communis* female was used.

For analysis, we computed the number of probing events and the number of encounters as frequencies over the allotted time (i.e. maximum of 5 min or until successful oviposition) (Desneux *et al.*, 2004). We compared these frequencies and the time until oviposition for the various aphid developmental stages using a non-parametric Kruskal-Wallis test. Next, pair-wise Mann-Whitney *U* tests were carried out following a Bonferroni correction for multiple comparisons. Proportional measures of attack and successful oviposition were compared between the different *A. glycines* stages using a Chi-square test. The same analysis was used to compare the proportion of aphids exhibiting defensive behaviours among the various stages.

Choice assay of host acceptance

A third experiment was done to determine whether *B. communis* prefers certain *A. glycines* stages over others and if such preference changes as a parasitoid forages within a patch of aphids of various stages. As in the previous assay, a soybean leaflet was placed upside down within a 5.8 cm dia. Petri dish filled with moist sand and placed under a dissecting microscope. On this leaflet (which will be referred to as the 'patch'), we placed a total of five individuals of each of the seven different developmental stages of *A. glycines*, totaling 35 aphids. We allowed the aphids to establish for 5–10 min and then introduced one *B. communis* female. Upon introduction of the parasitoid, the Petri dish was covered with a plastic lid 5.1 cm in diameter and 1.3 cm in height.

We noted the sequence of aphids that were encountered and recorded parasitoid attack and oviposition on each aphid attacked. The observation was terminated when the parasitoid stayed outside the patch for longer than 1 min or when 30 min had elapsed. As *B. communis* did not appear to discriminate against previously-parasitized aphids (see Results), we did not replace parasitized aphids during the course of the observations or treat them differently in subsequent data analysis. However, for each replicate, we composed a new patch using only unparasitized aphids collected from the *A. glycines* colony. The exposure was replicated 25 times.

Procedures for statistical analysis of this experiment were modified from Weisser (1994). Instar preference was measured using the formula of Manly (1974), whereby preference is scored as a deviation of the number of individuals of a given developmental stage selected for a particular behaviour (i.e. encounter, attack and oviposition) from the number of this stage eligible for the action (e.g. number present in the patch, number attacked, etc.). Let A_i be the number of individuals of a given stage i eligible for a particular action by the parasitoid ($\sum_{i=1}^{7} A_i = N =$ total number eligible for this action), and let x_i be the number of stage i that have been selected for this

Table 1. Life history traits of *Binodoxys communis* progeny emerging from the various developmental stages of its aphid host, *Aphis glycines*. Only one aphid stage was exposed to each adult female parasitoid in this experiment.

Host instar	Life history trait					
	Number of mummies formed	Proportion (of mummified aphids) emerged	Sex ratio (proportion males)	Days until mummification	Female development time	Male development time
1	7.22 + 2.13a	0.51 + 0.10a	0.56 + 0.14ab	7.00 + 0.12ab	11.25 + 0.44a	10.15 + 0.08a
2	10.66 + 1.85a	0.62 + 0.06a	0.69 + 0.09b	7.25 + 0.16b	11.45 + 0.33ab	11.23 + 0.28b
3	12.60 + 1.62a	0.73 + 0.07a	0.59 + 0.11ab	6.79 + 0.12a	11.77 + 0.29ab	11.95 + 0.22cd
4	16.40 + 2.81a	0.68 + 0.06a	0.42 + 0.11ab	7.07 + 0.14ab	12.12 + 0.24b	12.16 + 0.23d
Apterous adult	10.56 + 2.90a	0.61 + 0.10a	0.34 + 0.08a	6.94 + 0.11ab	11.27 + 0.13a	11.41 + 0.13bc
Alatoid 4th instar	9.80 + 2.14a	0.53 + 0.09a	0.48 + 0.12ab	6.80 + 0.12a	12.59 + 0.29bc	12.88 + 0.44de
Alate adult	15.70 + 5.37a	0.59 + 0.06a	0.56 + 0.11ab	8.40 + 0.15c	13.00 + 0.26c	13.03 + 0.26e

Mean ± SE; values within the same column followed by identical letters are not significantly different ($P > 0.05$, one-way ANOVA with Fisher's protected LSD).

particular action and r_i the number that have not been selected (so that $x_i + r_i = A_i$). We considered the case in which an aphid already selected for an interaction is still eligible for this action (Weisser, 1994).

Then

$$\beta j = \frac{(xj/Aj)}{\sum_{i=1}^{7} xi/Ai} \qquad j = 1,\ldots,7$$

is Manly's Beta of the *j*th stage for this particular action (with a total of seven different stages being considered). If βj is greater than 1/7 for any given developmental stage *j*, then the parasitoid prefers this given stage for the action under consideration. If βj is less than 1/7 then the parasitoid avoids this interaction with stage *j* and, finally, if $\beta j = 1/7$, then the parasitoids accept any eligible stage for the action under consideration. This formula is used to determine whether the different stages are encountered, attacked and parasitized in the same proportion as they are present within the patch. To determine whether *B. communis* preference changes with respect to the sequence of aphid attacks, we computed Manly's Beta values for different intervals over the course of the experiment (encounters 0–20, 20–40 and 40–60). We then compared these values between intervals for a select set of actions on each aphid stage (i.e. encounters, attacks or oviposition).

We compared Manly's Beta values for the different *A. glycines* stages using a Kruskal-Wallis test. For data that were normally distributed or could be successfully transformed, a One-way ANOVA was used, followed by Fisher's protected LSD as post-hoc analysis. All statistical analyses were executed using SPSS software (Landau & Everitt, 2004). For datasets that yielded non-significant differences, we performed a power analysis using GPower 3.0.4. (Faul *et al.*, 2007).

Results

No-choice parasitism trials

Binodoxys communis was able to successfully parasitize and develop on each of the seven stages of *A. glycines*. The number of mummies formed in each of the stages did not show any significant differences (table 1; ANOVA, $F_{6,62} = 1.05$, $P = 0.40$). However, the achieved power of this analysis was 0.46. Emergence rates of *B. communis* on the different *A. glycines* stages also did not show significant
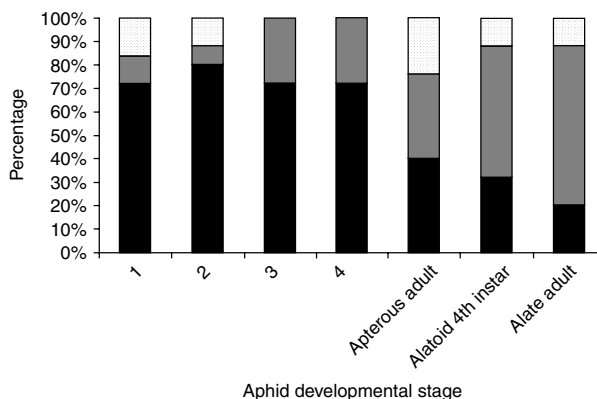

Fig. 1. Outcome of *B. communis* encounters with the various *A. glycines* developmental stages in a no-choice experiment. The graph represents the proportion of aphids (out of 25) of a given stage that were attacked or accepted for oviposition by *B. communis* (□, No attack; ▨, Unsuccessful attack; ■, oviposition).

differences (ANOVA, $F_{6,60} = 0.91$, $P = 0.50$). The power of this analysis was also low, equalling 0.37. Among the 4th instar alatoid nymphs that developed into mummies, 54.01 ± 0.08% (average ± SE) had transformed into winged adults prior to mummification, and the *B. communis* mummies produced from these also possessed wings.

The sex ratio of *B. communis* was highly male-biased on 2nd instar aphids, while female-biased on apterous *A. glycines* adults (table 1). Development time to mummification varied significantly with *A. glycines* stage (Kruskal-Wallis statistic = 92.72, $P < 0.001$) with mummification taking longest for alate adults. Time to emergence of both female and male *B. communis* also differed among *A. glycines* stages (Kruskal-Wallis statistic = 40.41, $P < 0.001$; KW statistic = 64.27, $P < 0.001$, respectively). In the various *A. glycines* stages, parasitoid development time gradually increased with aphid stage up until 4th instar. Parasitoids took longest to develop on alatoid nymphs and alate adults.

No-choice assays of host acceptance

Although attack by *B. communis* females did not differ among the various *A. glycines* stages in no-choice assays

Table 2. Acceptance behaviour of *B. communis* upon encounter with different *A. glycines* developmental stages in a no-choice experiment. Behavioural parameters are reported for all replicates ($N = 25$) and for wasps that successfully oviposited in the aphids presented. Number of probing events and number of encounters are indicated as frequencies over the allotted time (i.e. 5 min or until successful oviposition). Parameters include the total number of probing events or encounters and the total time until oviposition.

All replicates ($N = 25$)		
Host stage	Number of encounters (min^{-1})	Number of probing events (min^{-1})
1	3.27 ± 0.47a*	3.88 ± 0.60a
2	2.34 ± 0.26b	3.49 ± 0.58ab
3	2.27 ± 0.33b	3.99 ± 0.60a
4	2.16 ± 0.23bc	3.70 ± 0.57ab
Apterous adult	1.79 ± 0.31bc	2.24 ± 0.53bc
Alatoid 4th instar	1.77 ± 0.19bc	2.24 ± 0.64bc
Alate adult	1.39 ± 0.12c	1.70 ± 0.32c

Observations where successful oviposition was recorded				
Host stage	N	Number of encounters (min^{-1})	Number of probing events (min^{-1})	Time until oviposition (s)
1	18	3.97 ± 0.55a	2.25 ± 0.12a	34.00 ± 8.04a
2	20	2.69 ± 0.26b	1.98 ± 0.14a	58.90 ± 12.21a
3	18	2.81 ± 0.38b	2.08 ± 0.17a	71.83 ± 18.37a
4	18	2.50 ± 0.27b	2.10 ± 0.15a	70.22 ± 12.93a
Apterous adult	10	2.91 ± 0.59ab	2.12 ± 0.17a	69.10 ± 26.13ab
Alatoid 4th instar	8	2.25 ± 0.31b	2.19 ± 0.31a	129.75 ± 25.66c
Alate adult	5	1.65 ± 0.42b	1.83 ± 0.08a	136.80 ± 32.42bc

Mean $\pm$ SE; values within the same column followed by identical letters are not significantly different ($P > 0.05$, Mann-Whitney U test).
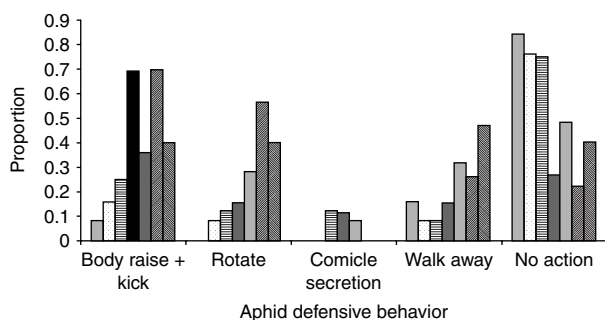

Fig. 2. Aphid defensive behaviours associated with *B. communis* attacking the various developmental stages of the soybean aphid, *Aphis glycines*, in a no-choice experiment. The proportion of aphids (out of 25) of a given instar exhibiting certain behaviours is indicated (☐, 1; ☐, 2; ▨, 3; ■, 4; ▨, Adult; ▨, Alatoid; ▨, Alate).

(fig. 1; likelihood ratio $\chi^2 = 1.23$, $P = 0.97$), parasitoid oviposition success did vary with stage (likelihood ratio $\chi^2 = 15.65$, $P = 0.02$). Attacks on apterous adult, alate and alatoid nymphal stages were less likely to succeed than attacks on the apterous nymphal stages. Based on all replicates, we found *B. communis* to encounter 1st instars more frequently than other stages and alate adults less frequently than 2nd instars (table 2; Kruskal-Wallis statistic = 16.26, $P = 0.01$). Also, parasitoids probed immature *A. glycines* stages more frequently than alatoids and adults (Kruskal-Wallis statistic = 18.284, $P = 0.006$). First instar *A. glycines* were probed more frequently than other stages. The preferred location for oviposition was the aphid thorax, receiving 70.1% of successful ovipositions, compared to 17.5% for the head region and 12.4% for the abdomen. Aphid instars 1–4 received 72, 65, 72 and 66% of ovipositions in the thorax and 0, 5, 22 and 33% in the head region, respectively. Apterous

adults, alatoids and alates received 80, 50 and 100% of oviposition in the thorax and 20, 50 and 0% in the head region, respectively.

The various *A. glycines* stages differed in their defensive behaviour (fig. 2). Many of the immature aphid stages did not exhibit any defensive behaviour, and the frequency of inaction varied among *A. glycines* stages (Chi-square $\chi^2 = 16.348$, $P = 0.012 < 0.05$). Kicking was the most frequently recorded behaviour (seen in 38% of the aphids). The frequency of kicking or body rotation varied among aphid stages (Chi-square $\chi^2 = 21.818$, $P = 0.001$; $\chi^2 = 24.718$, $P < 0.001$, respectively). Some aphids exhibited more than one type of defensive behaviour, commonly combining kicking with body rotation.

Choice assay of host acceptance

Parasitoid wasps stayed within the patch for 21.64 ± 1.40 min and encountered 43.80 ± 3.67 (average $\pm$ SE) aphids, indicating that aphids were frequently encountered more than once. *Binodoxys communis* encountered the different *A. glycines* stages to varying extents (fig. 3; ANOVA $F_{6,168} = 5.71$, $P < 0.001$). There were also significant differences in attack rates among stages that were encountered and in oviposition rates among stages that were attacked (fig. 3; ANOVA $F_{6,168} = 9.76$, $P < 0.001$; Kruskal-Wallis $\chi^2 = 74.61$, $P < 0.001$, respectively). Attack rates were highest for the young instars and lowest for alate and apterous adults, given their respective encounter rates. Lastly, 1st and 2nd instar *A. glycines* were also oviposited to highest extent, given their respective rates of attack.

Parasitoids did not alter their preference for oviposition of certain aphid stages during the course of the experiment. Manly's Beta values for this action did not differ between the three intervals (Kruskal-Wallis statistic = 1.88, $P = 0.39$; Kruskal-Wallis statistic = 1.60, $P = 0.45$; Kruskal-Wallis statistic = 3.95, $P = 0.14$; Kruskal-Wallis statistic = 0.53,
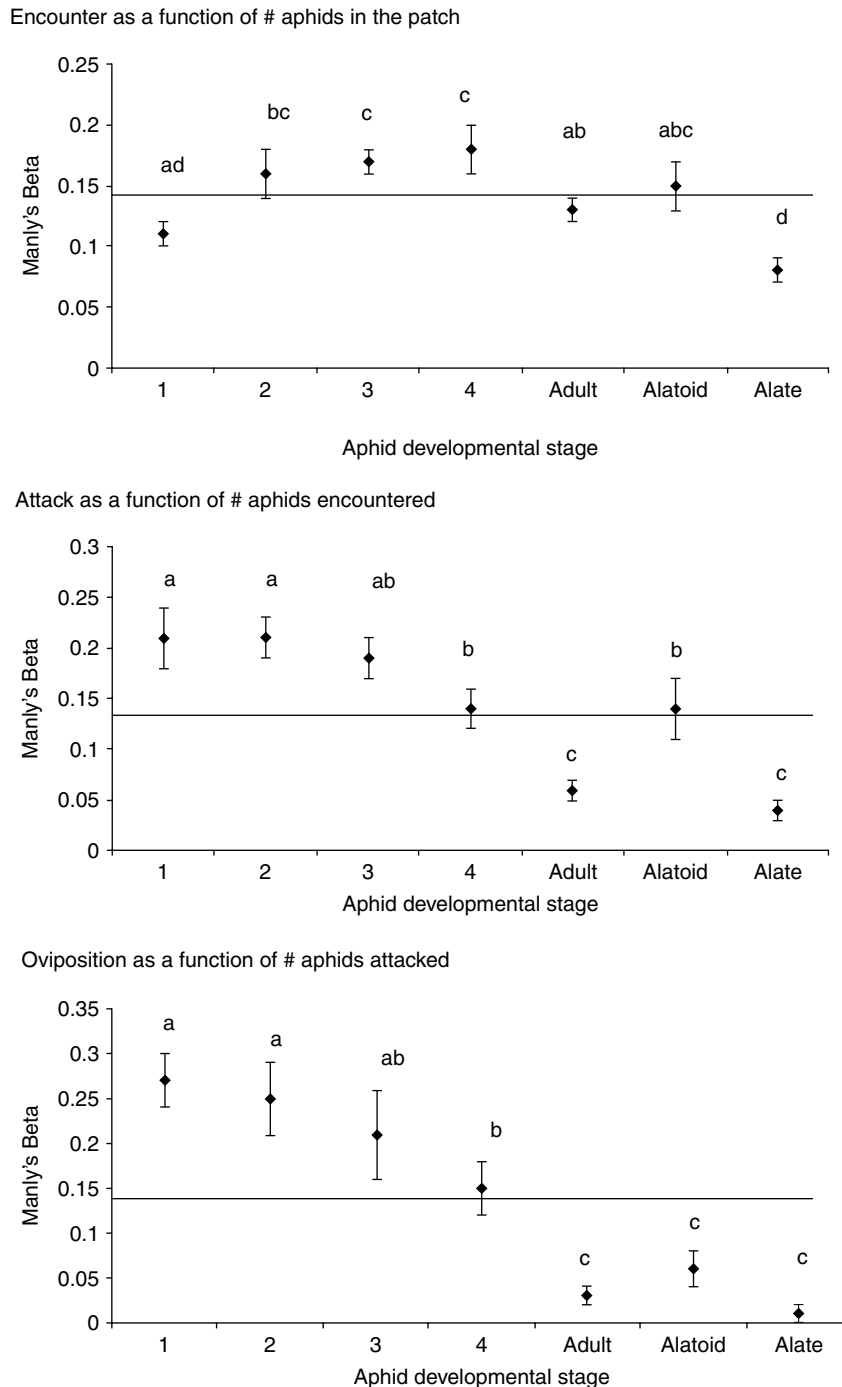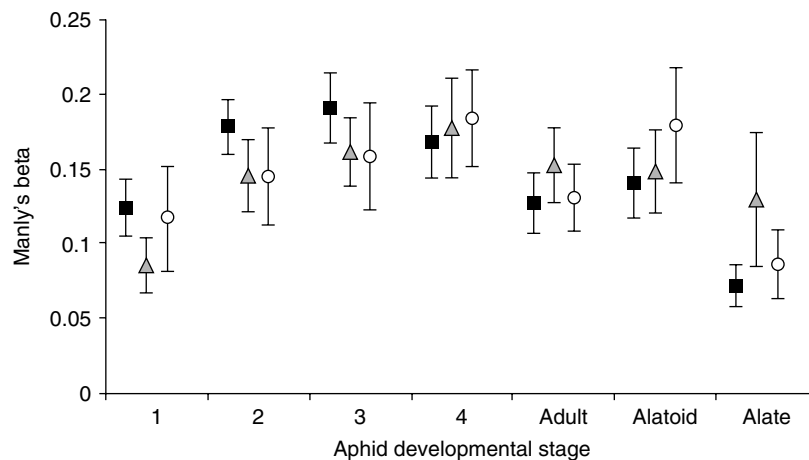
Encounter as a function of # aphids in the patch

Attack as a function of # aphids encountered

Oviposition as a function of # aphids attacked

Fig. 3. Average (±SE) Manly's Beta values for *B. communis* behaviour in patches with a total of 35 *A. glycines* individuals, equally distributed among all seven different developmental stages. Within each figure, the line represent the baseline value (1/7) against which Manly's Beta values for each stage are compared. Different letters indicate significantly different Manly's Beta values for the subsequent aphid developmental stages ($P > 0.05$, one-way ANOVA with Fisher's protected LSD post hoc analysis).

$P = 0.77$; Kruskal-Wallis statistic $= 0.30$, $P = 0.86$; Kruskal-Wallis statistic $= 1.98$, $P = 0.37$; Kruskal-Wallis statistic $= 1.86$, $P = 0.39$ for 1st, 2nd, 3rd, 4th instar, adult, alatoid and alate stages, respectively). Also, *B. communis* did not modify its preference for encounter or attack of any of the aphid stages over the allotted 30 min period (fig. 4).

Discussion

Many parasitoids in the braconid subfamily, Aphidiinae, preferentially parasitize small or intermediate host instars (Liu *et al.*, 1984; Sequeira & Mackauer, 1987, 1992a; Weisser, 1994; Mackauer *et al.*, 1996; Ives *et al.*, 1999; Sharmila &

Encounter as a function of # aphids in the patch

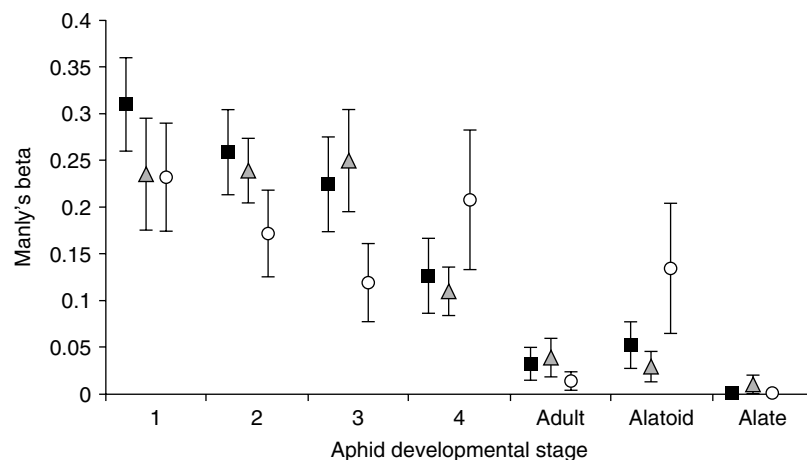
Oviposition as a function of # aphids encountered

Fig. 4. Variation in *B. communis* preference for the different *A. glycines* developmental stages over the course of a 30 min choice experiment. Parasitoid preference for a given stage is represented as Manly's Beta values ($\pm$SE), which indicate whether the various stages are either encountered in the same proportion as they are present within the patch or oviposited in the same proportion as they are encountered. Values are computed for the following intervals: encounters 0–20 (■), 20–40 (△) and 40–60 (○).

Rajendra, 1999; Chau & Mackauer, 2000, 2001; Perdikis *et al.*, 2004). Our research confirms this general pattern for *B. communis*, a parasitoid of the soybean aphid, *A. glycines*. No-choice assays showed a high proportion of successful attacks on all apterous nymphal *A. glycines* instars, while choice trials indicated lower encounter, attack and oviposition of apterous and alate adults, as well as alatoid nymphs. Nevertheless, parasitism trials with exposures over a longer time revealed similar *B. communis* parasitization of the various *A. glycines* stages. This disparity could hint at a lower suitability of young *A. glycines* instars for development of *B. communis*, as indicated below.

Parasitism of the various *A. glycines* stages possibly has major implications for fitness of *B. communis* offspring. Parasitism levels, mummy emergence and parasitoid sex ratio showed little differences among the various aphid stages. However, *B. communis* showed a higher rate of acceptance of young *A. glycines* instars compared to adults or

alatoid nymphs. Thus, younger instars may have experienced greater mortality following parasitism (Rakhshani *et al.*, 2004; Colinet *et al.*, 2005). Alternative explanations are that super-parasitism levels of preferred younger instars is high or that host-stage preferences are not expressed in patches containing a single host stage, particularly for naïve parasitoids. Lastly, additional time of exposure (4 h) during parasitism trials could lead to higher parasitism rates of older stages despite short-term behavioural avoidance.

With the exception of the *A. glycines* apterous adult stage, development time of both *B. communis* sexes increased with aphid stage. Various relationships exist between parasitoid development time and host age at oviposition (Hopper, 1986; Colinet *et al.*, 2005), with positive associations being occasionally reported (Vinson, 1972; Lawrence *et al.*, 1976). Rapid parasitoid development in 1st instar *A. glycines* shows that these hosts provide minimum required nutrient

levels for *B. communis* (Henry *et al.*, 2005) although parasitoids emerging from young hosts may be smaller. The gradual increase in development time on later *A. glycines* stages may reflect changes in nutritional value of the host, increased aphid resistance and competition of parasitoid larvae with the developing host embryos (Walker & Hoy, 2003; Colinet *et al.*, 2005) or increased time necessary for development of a larger parasitoid. No evidence was found of delayed parasitoid development in younger or smaller hosts, a common pattern in koinobiont parasitoids (Harvey, 2005). Younger *A. glycines* instars were much smaller than later developmental stages (Hodgson *et al.*, 2005; K. Wyckhuys, personal observation).

The interaction between *B. communis* and *A. glycines* is also mediated by host behaviour, particularly aphid defense. In no-choice assays, *A. glycines* exhibited a variety of defensive behaviours, all of which are commonly observed among aphid species (e.g. Gerling *et al.*, 1990; Hågvar & Hofsvang, 1991; De Farias & Hopper, 1999; Villagra *et al.*, 2002). In no-choice assays, *B. communis* did not refrain from attacking larger or older aphid stages or aphids that exhibited stronger defenses. This could reflect either a low response threshold of *B. communis* for oviposition (Mackauer *et al.*, 1996) or acceptance decisions resulting from its lack of previous experience (Henry *et al.*, 2005). *Binodoxys communis* females encountered and probed larger aphid stages at a lower frequency and with many probing attempts unsuccessful. Like other members of the genera *Trioxys* and *Binodoxys*, *B. communis* uses a pair of terminal abdominal prongs to grasp the host prior to oviposition (Völkl & Mackauer, 2000), and this grasping is thought to be more effective on smaller instars. Also, as older *A. glycines* stages were less frequently oviposited in and exhibited more body rotation and walking behaviours, thus these defensive behaviours may deter *B. communis* attack. However, 4th instar apterous *A. glycines* exhibited kicking behaviour as frequently as 4th instar alatoid nymphs; but the former were oviposited in as often as younger instar apterous nymphs, suggesting that this defense does not always work (see fig. 1).

In choice assays, *B. communis* females encountered alate morphs and 1st instars less often than other stages and morphs, while encountering 3rd and 4th instars at a higher rate than other stages and morphs. Although most parasitoids have poor ability to assess host suitability from a distance, they sometimes evaluate aphid shape, size or movement (Battaglia *et al.*, 1995; Le Ralec *et al.*, 2005). Our results suggest that *B. communis* might employ aphid size as an initial criterion to determine host suitability, while increased movement of *A. glycines* alates may act as a release stimulus for attack.

Because *B. communis* successfully develops on all *A. glycines* developmental stages, field releases do not need to target specific phases of aphid infestation. Also, considering that young aphid instars are generally more abundant than older stages in field populations (Hughes, 1963; Chau & Mackauer, 1997; Losey & Denno, 1998), parasitoids are very likely to successfully establish irrespective of *A. glycines* colony composition. Parasitoid preference for younger stages can significantly affect host population growth (e.g. Lin & Ives, 2003), while a sustained attack of older and larger *A. glycines* stages, along with its induction of costly defenses could reduce reproductive capacity of *B. communis* (Nelson & Rosenheim, 2005).

Successful *B. communis* parasitism of *A. glycines* alatoid nymphs and alates and its increased development time on winged aphid hosts suggests the existence of a phoretic association. Such association was suggested by Hoelmer & Kirk (2005), who reported the presence of *B. communis* in association with *A. glycines* at early stages of their colonization of soybean fields in China and hypothesized parasitoid arrival as eggs carried within winged aphids (see also Liu *et al.*, 2004). Also, the finding that *B. communis* does not disrupt the development of wings of *A. glycines* alatoid nymphs (see Demmon *et al.*, 2004; Christiansen-Weniger & Hardie, 2000) indicates that flight of parasitized aphids might be possible. Our findings can have implications for parasitoid establishment, dispersal capability and biological control success upon release in novel environments.

Acknowledgements

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